REMARKS

This Reply is responsive to the Office Action dated April 24, 2001. Entry of the foregoing and reconsideration on the merits pursuant to 37 CFR 1.112 is respectfully requested.

The application has been amended as set forth above. In accordance for the new rules for amending applications set forth in 37 CFR 1.121, which took effect on March 1, 2001, a marked up version showing all amendments is attached hereto as an appendix.

Specifically, the specification has been amended at page 9 to correlate the microsatellite linkage map represented in Figure 2 with the relevant description in the specification.

Claim 3 has been amended to preserve antecedent basis with Claim 1 and to clarify that the Z-chromosomal DNA used in the method of Claim 3 is a marker DNA.

Claim 4 has been amended to specify that the Z-chromosomal specific DNA map generated in the claimed method is a microsatellite DNA linkage map. Support for this amendment may be found in the specification at page 5, lines 5-18.

Claim 6 has been amended to clarify that gross chromosomal rearrangements are identified by comparing two Z-chromosome specific microsatellite linkage maps. Support for this amendment may be found in the specification at the very least at page 3, lines 17-20. No new matter was added by any of these amendments.

In addition, new claims 8 and 9 were added. Claim 8 further limits Claim 4 and specifies that the Z-chromosome specific microsatellite linkage map may be generated by confirming the presence and location of said marker DNA on the Z-chromosome using PCR amplification and/or fluorescent *in situ* hybridization (FISH). Support for this claim may be found in the specification at page 9, lines 12-14 and page 9, lines 19-20.

Claim 9 further limits Claim 6 and specifies that the second Z-chromosome specific microsatellite linkage map is generated using a species of avian that is different from the first, and that said second map is generated using heterologous fluorescent *in situ* hybridization (FISH) of metaphase chromosomes. Support for this claim may be found in the specification at page 7, lines 17-18, and at page 8, lines 13-16. No new matter has been added by way of the newly submitted claims.

Turning now to the Office Action, the Examiner notes on page 2 of the Office Action that no CRF was located in the priority provisional application. Accordingly, a CRF is

submitted herewith, along with the requisite statement that the CRF and the paper copy previously submitted on April 21, 2000, are the same.

Claims 1-7 were rejected under 35 U.S.C. §112, first paragraph for containing subject matter that was allegedly not enabled by the specification. In particular, the Examiner stressed that the amount of experimentation in order to practice the invention would be profound, with little if any reasonable expectation of success; that the specification merely invites experimentation as to determining the significance of the isolated sequences; that the specification does not teach development of a genetic map for avian species; that the art relating to the genome of avians is undeveloped; and that the specification does not enable use of the disclosed sequences. Applicants respectfully traverse each ground for the rejection.

First, the Examiner alleges that the amount of experimentation required to practice the invention would be "profound" and has "little if any reasonable expectation of success." Applicants respectfully submit that this analysis is in error, because the specification clearly discloses the marker sequences recited in Claim 1 by sequence, and describes their use in methods to generate an avian microsatellite linkage map using techniques that were known in the art at the time the invention was filed (see Figure 2). Applicants have also described the use of the claimed sequences as labeled heterologous painting probes in FISH analysis of turkey metaphase chromosomes, in order to show that such sequences can be used to generate a corresponding microsatellite map of another avian species, which map may be used in comparative genomics in order to identify chromosomal rearrangements existing in the Z-chromosomes between the two species (see the paragraph bridging pages 7-8). Thus, Applicants fail to understand how a profound amount of experimentation is required when Applicants have already demonstrated successful operation of the claimed methods using the chicken and turkey genomes.

The Examiner also argues that the specification is at best an invitation to experiment in order to determine the significance of the isolated sequences. However, the Examiner appears to overlook the fact that the claimed sequences find significance as chromosomal markers, for use in a wide variety of mapping activities. Applicants believe the significance of the claimed DNAs was emphasized in the application as filed, in that original Claim 1 clearly refers to the claimed DNAs as "markers." The significance of the DNAs is further emphasized by Claim 2, taken in the context of Figure 2. Indeed, the microsatellite markers

are significant at the very least in that together they form a library representing a microsatellite map of the avian Z-chromosome.

The Examiner appears to be arguing that the DNA sequences find significance only in the determination of the genes they encode, and this is simply not true. It is correct that one use of the marker DNAs is to identify and/or map the location of genes encoding desirable traits and for identifying sex-linked genotypes (see the specification at page 10, lines 5-7). Indeed, as discussed in the attached abstract of the article by Reguigne-Arnould et al. (Genomics, 1996, 32(3): 458-61), microsatellite markers have been used as tools for the localization of disease-related genes. Ye et al. (Genomics, 1995, 28(3): 566-69) used microsatellite marker DNAs to map the location of the Werner Syndrome (WRN) gene (abstract attached). However, this is not the only significance of such sequences.

As noted in the specification at page 10, lines 3-5 and lines 10-12, and as exemplified through the working examples of the specification, microsatellite markers are useful for genetic mapping and for comparative chromosomal analyses pertinent to the evolution of species. In the present specification, Applicants have used the claimed sequences to generate a microsatellite linkage map of the chicken Z-chromosome (Figure 2), and this map was compared to a similar map generated for the turkey Z-chromosome using "heterologous painting" as described at the bottom of page 7. When the same marker sequences are labeled and used to paint the corresponding chromosome in another avian genome, gross deletions and rearrangements occurring between the two species' Z chromosomes may be identified.

Using microsatellite linkage maps to analyze genetic variability is not new, therefore, Applicants fail to understand why the Examiner believes that the art relating to the claimed methodology is undeveloped. For instance, Jiang et al. (Mamm. Genome, 1995, 6(9): 586-91) used microsatellite mapping to analyze genetic variability at the murine H2 locus. Miura and Nakamura (Nippon Rinsho, 1996, 54(4): 986-91) used microsatellite markers to identify chromosomal deletions relating to esophageal squamous cell carcinoma. And Ponce de Leon et al. (Proc. Natl. Acad. Sci. USA 93: 3450-54) showed that similar microsatellite painting probes could be used to compare homologies between the X chromosomes of various mammalian species (paper attached). While the Examiner is correct to note that the art pertaining to avian genomics is not as developed as that pertaining to mammalian species, resolving this long felt need was in fact a goal of the present invention. Indeed, by providing a series of microsatellite markers of the avian Z-chromosome, the present invention enables a

variety of mapping analyses that will advance the state of the art pertaining to avian genomics.

The Examiner apparently dismisses Applicants' painting experiments relating to the turkey genome because it is allegedly unclear as to what target sequence the sequences are binding. This misses the point, which was that the probes could be used to study whether the same microsatellite linkage map is observed in closely related species, in order to detect whether any gross chromosomal rearrangements occurred during the progression of evolution. The Examiner states specifically at page 5 of the Office Action that "a review of the specification fails to find where a genetic map has been developed." Applicants respectfully note that a genetic microsatellite linkage map for the chicken Z chromosome showing the location of each of the marker DNAs is depicted in Figure 2.

The Examiner also asserts on page 6 of the Office Action that the specification fails to enable the use of the claimed sequences, and points with particularity to the discussion at page 8 where Applicants note that it is *expected* that the painting probes can be similarly used in identifying chromosomal arrangements in other species. Applicants respectfully note that the expectation that the probes could be used to study the Z chromosomes of species other than the chicken and the turkey is entirely valid given the annealing of the chicken marker sequences to turkey genomic DNA. Applicants' pointing out that such a use stands a reasonable expectation of success should not be taken in isolation as an admission that the invention has not been reduced to practice. Indeed, by pointing to this passage in isolation the Examiner appears to overlook the microsatellite map in Figure 2 generated by Applicants using the claimed sequences, and the disclosure that the microsatellite DNAs were successfully employed as painting probes in an analysis of the turkey Z chromosome.

For all the above reasons, reconsideration and withdrawal of the enablement rejection under the first paragraph of §112 is respectfully requested.

Next, Claims 3-7 were rejected under 35 U.S.C. §112, second paragraph, as allegedly omitting essential steps. Applicants respectfully traverse. According to MPEP 2172.01, essential steps are those that are necessary to practice the invention. As a method of genomic mapping employing the marker sequences recited in Claim 1 may be practiced different ways, there are no absolutely necessary steps. For instance, a microsatellite linkage map is formed by locating the position of the marker sequences on the chromosome in relation to one another. This can be done by a variety of ways, including PCR amplification and FISH

analysis. There is no one set way to identify the location of the marker sequences on the chromosome in relation to one another. Hence, there are no absolutely essential steps to the claimed methods. Nevertheless, Applicants note that new claims 8 and 9 have been added, which further define the manner in which the microsatellite map may be generated, and define one way in which comparative genomic analysis may be conducted in order to detect gross chromosomal rearrangements, respectively. Reconsideration and withdrawal of the rejection is respectfully requested.

Finally, Claims 1-7 were rejected under 35 U.S.C. §101 because the claimed invention is allegedly not supported by a specific or substantial utility or a well-established utility. According to the Office Action, the Claims 1 and 2 are drawn to Z-chromosomal markers that need not be isolated, and for which the amino acids encoded have not been defined. Therefore, it is alleged that the only utility for the sequences is in developing genetic maps of avian species, and the utility of such maps is doubted. Further, the Examiner alleges that such maps were not in Applicants' possession at the time of filing. Applicants respectfully traverse.

First, Applicants respectfully note that Claim 1 has been amended to indicate that the sequences are "isolated." However, this should not be taken to mean that the actual nucleotide sequences must be physically isolated for those of skill in the art to use such sequences in mapping experiments. Indeed, one of skill in the art could design a probe or primer based on the disclosed sequences or part of the disclosed sequences, and use such a probe as a painting probe, for instance, in order to study gross chromosomal rearrangements among species.

Second, Applicants respectfully note again that the specification does report experiments whereby the physical location of the microsatellite marker sequences was determined and used to create a microsatellite linkage map (see Figure 2). Therefore, contrary to what is asserted in the Office Action, Applicants were in possession of a genetic microsatellite map at the time of fling.

Finally, Applicants respectfully submit that the map disclosed in the present invention does indeed have specific and substantial utility, as other groups who have since used similar mapping techniques would surely agree. Again, as noted in the specification at page 10, lines 3-5 and lines 10-12, and as exemplified through the working examples of the specification, microsatellite markers are useful for genetic mapping and for comparative chromosomal

analyses pertinent to the evolution of species. In the present specification, Applicants have used the claimed sequences to generate a microsatellite linkage map of the chicken Z-chromosome (Figure 2), and this map was compared to a similar map generated for the turkey Z-chromosome using "heterologous painting" as described at the bottom of page 7. When the same marker sequences are labeled and used to paint the corresponding chromosome in another avian species, gross deletions and rearrangements occurring between the two species' Z chromosomes may be identified.

Other groups have used since used similar techniques to conduct genomic analyses, thereby confirming the established utility of the methods disclosed herein. For instance, Nishida-Umehara et al. (Chromosome Res., 1999, 7(8): 635-40) used FISH with three probes linked either to Z or W chromosome in most avian species examined thus far and concluded that structural rearrangements such as deletions and inversions might have been the initial step of W chromosome differentiation from an ancestral homomorphic pair in the cassowary (see attached abstract). Groenen et al. (Genomics, 1998, 49(2): 265-74) used a microsatellite linkage map of the chicken genome to identify two new regions of conserved synteny between human and chicken and confirm other previously identified regions of conserved synteny between human and chicken (see attached abstract). And as discussed by Ruyter-Spira et al. (Anim. Genet., 1998, 29(2): 85-90), microsatellite markers are tools for adding expressed sequence tags to the genetic linkage map of the chicken.

Finally, Applicants respectfully request that the Examiner review the Groenen et al., 2000 reference attached hereto (Genome Res., 2000, 10(1): 137-47), which reviews how the microsatellite markers of different groups have been combined to form a detailed linkage map of the chicken genome. The reference also discusses some of the reasons why information regarding the chicken genome is of significant utility. For instance, according to this reference, the chicken is increasingly becoming of great interest as an intermediate evolutionary model organism because of its smaller genome size, the low amount of repetitive sequences and reduced intron sizes in its genome, and particularly because the level of conserved synteny between chicken and humans appears to be very high. The chicken is also being studied intensively for genes affecting polygenic traits (quantitative trait loci or QTL), which has contributed to the international efforts toward detailed physical and linkage mapping in the chicken.

Thus, Applicants respectfully submit that the microsatellite markers of the present invention have specific and substantial utility in generating a microsatellite linkage map, and that such a map has specific and substantial utility in contributing to a detailed map of the chicken genome, and in serving as a tool for comparative genomic analyses with other avian genomes. Such specific and substantial utility has since been confirmed by the international efforts reviewed in the Groenen reference attached hereto, and is now well-established. Reconsideration and withdrawal of the rejection under §101 is therefore respectively requested.

All issues raised by the Office Action dated April 24, 2001, have been addressed in this Reply. Accordingly, a Notice of Allowance is next in order. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that she contact the undersigned so that such issues may be addressed expeditiously.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached Appendix is captioned **"Version with markings to show changes made"**.

All objections and rejections having been addressed, it is respectfully submitted that the present application is in a condition for allowance and a Notice to that effect is earnestly solicited.

Respectfully submitted,

PILLSBURY WINTHROP LLP

By: _____

Bonnie D. Weiss
Registration No. 43,255

1600 Tysons Boulevard
McLean, VA 22102
(703) 905-2000
(703) 905-2500 Facsimile

Attorney Reference: 015837-0275805
Date: September 24, 2001

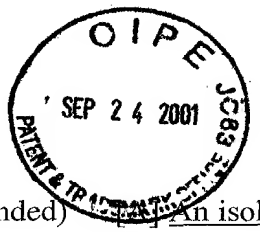
Enclosure: Appendix
Cited publication and abstracts

APPENDIX: VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

At page 9, the paragraph beginning at line 2:

Genetic and physical mapping of human and animal genomes has been greatly facilitated by the use of chromosome specific DNA libraries. Mapping with libraries specific to a chromosome or chromosomal region increases marker saturation by reducing the gaps resulting from a purely random shotgun approach. This study was undertaken to construct a genetic and physical map of microsatellites on the chicken Z chromosome. This chromosome is the fifth largest in the chicken genome, comprising about 8% of the total. Notwithstanding its size, very few microsatellites have been assigned to it. DNA originating from the chicken Z chromosome was previously isolated and reported. This was used to construct a small insert library in Lambda ZAP Express, representing 14 chromosome equivalents. This library was screened for microsatellites with an (AC) 12 oligo, and positive clones were isolated. Confirmation of the presence of the microsatellite, as well as its approximate location along the cloned fragment was accomplished by PCR amplification. Clones with adequate flanking regions were sequenced, and primers for 19 microsatellites were constructed. These primers were used to genotype individuals from the East Lansing Poultry Reference Population and a linkage map was constructed. Fourteen markers were scorable and polymorphic in this population. The resulting map contains 12 markers in two linkage groups spanning 90 Cm and two unlinked markers (see Fig. 2). The physical location of each marker was established by fluorescent in situ hybridization (FISH). Preliminary results with four markers allowed the assignment of one linkage group to the long arm of the Z chromosome, and one to the short arm.



In the Claims:

1. (Amended) [A] An isolated Z-chromosomal marker DNA selected from the group consisting of Sequence 1 (43.Seq), Sequence 2 (71.Seq), Sequence 3 (80.Seq), Sequence 4 (81.Seq), Sequence 5 (131.Seq), Sequence 6 (147.Seq), Sequence 7 (166.Seq), Sequence 8 (196.Seq), Sequence 9 (199.Seq), Sequence 10 (204.Seq), Sequence 11 (235.Seq), Sequence 12 (249.Seq), Sequence 13 (258.Seq), Sequence 14 (290.Seq), Sequence 15 (309.Seq), Sequence 16 (341.Seq), Sequence 17 (398.Seq), Sequence 18 (420.Seq), and Sequence 19 (435.Seq).

3. (Amended) A method of using at least one Z-chromosomal marker DNA according to Claim 1 for genetic mapping.

4. (Amended) The method of Claim 3, wherein the genetic mapping is effected to construct a Z-chromosome specific microsatellite DNA linkage map.

6. (Amended) The method of Claim 4, [which is used] wherein said at least one Z-chromosomal marker DNA is used to construct a second Z-chromosome specific microsatellite linkage map, and the two maps are compared in order to identify gross chromosomal rearrangements.